Evidence for the possible function of the fluorescent granular perithelial cells in brain as scavengers of high-molecular-weight waste products

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Summary. The fluorescent granular perithelium (FGP) of rats and humans under experimental and pathological conditions was examined with the electron microscope. The FGP incorporated high molecular-weight protein (ferritin) and carbon particles administered intraventricularly. In a case of spontaneous cerebral hemorrhage, the FGP was found to contain lipoidal products and minute fragmented cell debris. The FGP in a patient with lipidosis contained pale inclusion bodies. In aged individuals, the inclusion bodies formed irregular larger aggregates.

As reported previously, the fluorescent granular perithelial (FGP) cells in animals and humans are localized mainly in bifurcations of the small cerebral vessels[1,2]. The main role of FGP is uptake and digestion of endo- or exogenous substances in the central nervous system[3,4]. Although the appearance of FGP resembles that of the neurolipomastoid cells of Ibrahim[5], as Sturrock[6] has pointed out, the function of FGP is thought to be quite different from that of neurolipomastoid cells. In this paper, we present several observations essential for a discussion of the function of FGP cells.

Materials and methods. 9 Wistar rats, 5 months, 10 months and 3 years old were used in this study. One rat of each age was used for control. 6 rats were injected intraventricularly with 0.02 ml physiological saline containing 50 mg ferritin or India ink suspension (Günter Wagner, C 11/1431a), and 2 h after the injection, their brains were removed and put into cold physiological saline. 2 old rats suffering from spontaneous cerebral hemorrhage were also studied. The human specimens were collected from area 6 of the cerebral cortex of cadavers used for dissection practice and pathological examination. The specimens were prefixed with a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde solution (adjusted to pH 7.4 with 0.1 M phosphate buffer), and postfixed with 1% osmium tetroxide solution buffered with 0.1 M phosphate solution and embedded in Epon 812. After cutting with a Porter-Blum ultramicrotome, thin sections were observed with a JEM 100B electron microscope.

Observation. The observations consisted of the following 3 steps: The first step was aimed at describing the normal morphology of inclusion bodies in FGP and of the bifurcating regions of small blood vessels using rats (5 months old) and human specimens (20 years old) (figs. 1 and 2). In the second step, the incorporation of exogenous materials such as ferritin, carbon and fragmented cell debris was studied (figs. 3–6). Thirdly, the profiles and contents of inclusion bodies obtained from the human specimens with lipidosis (2 years old) and glioma (15 years old), and from a middle aged man with aortic aneurysm (54 years old) were observed (figs. 7–9).

Figures 1 and 2 show endothelium covering vascular wall and cross or oblique aspects of smooth muscle cells in the bifurcation of small blood vessels. The FGP cells were situated adjacent to the smooth muscle cells and contained several round, homogeneous, dense inclusion bodies. These are typical features of FGP of young rats and humans. In general, in cerebral blood vessels, the endothelial cells were flat and poor in vesicles, but, in the bifurcations, as seen in these figures, the endothelial cells were high and provided with long cytoplasmic projections, and pinocytotic vesicles were recognized in their cytoplasm.

The intraventricular administration of India ink was followed by a deposition of some carbon particles in the inclusion bodies of FGP (fig. 3), and no particles could be seen in the other cells – smooth muscle cells and astrocytic processes. This indicates that the FGP can take up solid small particles. After

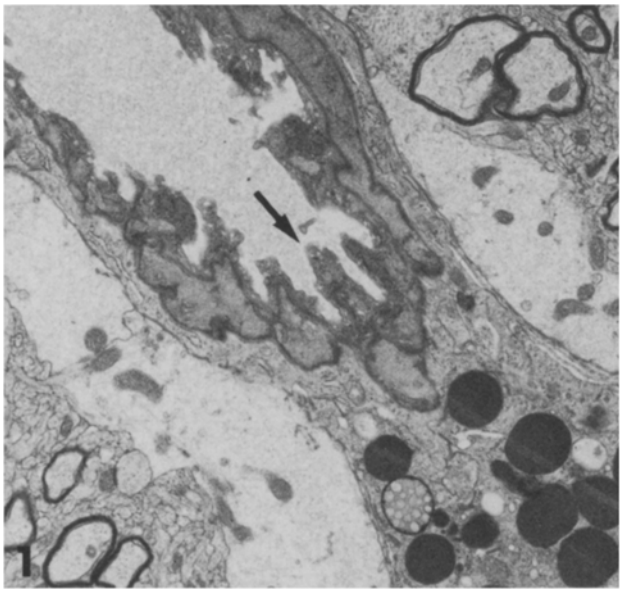


Figure 1. Bifurcating region of a small cerebral vessel. The cytoplasmic projections of endothelium are prominent (arrow). The FGP is seen between vascular wall and astrocyte, and involves typical round dense inclusion bodies. Rat, 5 months, control. × 5700.

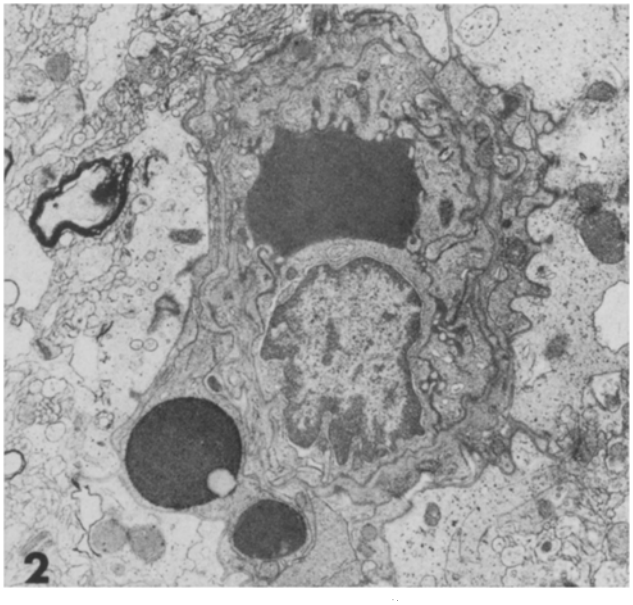


Figure 2. The same region as in figure 1. Endothelium is high and the arrangement of smooth muscle cells is complicated. The FGP possess round and dense inclusion bodies. Human, 20 years old, heart failure. × 7000.

the administration of ferritin, a lot of fine granules were clearly observed in the round dense inclusion bodies (fig. 4), but not in the honeycomb-like inclusion bodies.

In a case of spontaneous hemorrhage in the thalamus, numerous macrophages appeared close to bleeding areas. They contained many residual bodies and laminated structures. The remote FGP looked healthy and did not respond to the injury, judging from their appearance and contents (fig. 5). However, the FGP localized closer to the hemorrhage contained many pale inclusion bodies, small cellular fragments and lipoidal waste products enclosed with a dense rim (fig. 6). No myelin figures or large phagosomes could be found in them (a difference from macrophages). It means that the FGP remove minimum-sized cellular fragments and lipo-proteinous substances from the damaged central nervous system.

A peculiar feature was observed in the patient suffering from lipidosis (clinically diagnosed GM2). The cytoplasm of the FGP cells was occupied by large and pale inclusion bodies (fig. 7). The pale inclusion bodies could contain lipid or lipopolysaccharide, possibly associated with an accumulation of ganglioside in the FGP.

The next figure was taken from the region adjacent to a glioma. Inclusion bodies of FGP cells were vacuolated and spotted (fig. 8). Their electron opacity was heterogeneous, and

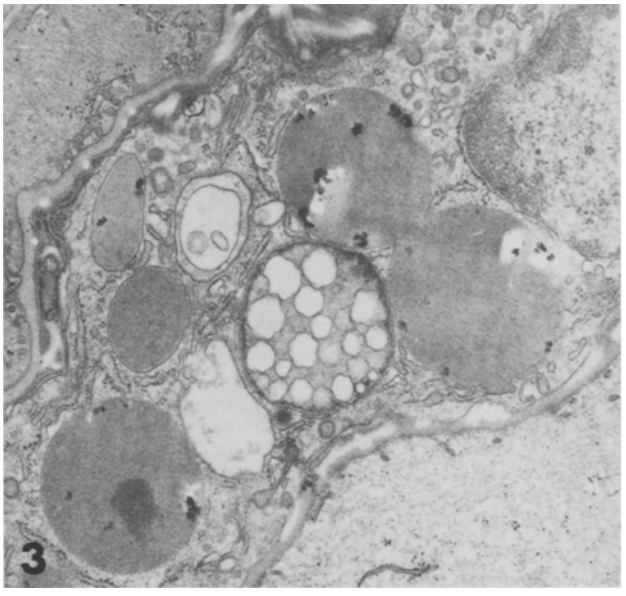


Figure 3. In dark inclusion bodies, several carbon particles are incorporated. Rat, 5 months, India ink injection. × 15,000.

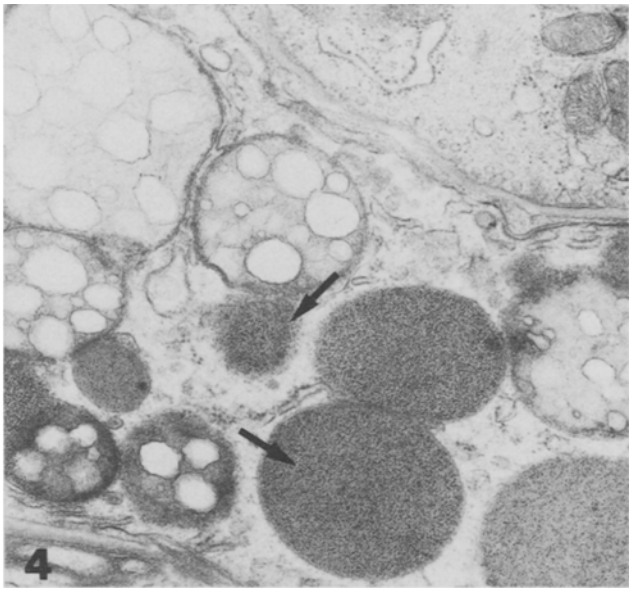


Figure 4. Fine granules are seen in dark inclusion bodies (arrows). No granules are present in honeycomb-like inclusion bodies. Rat, 10 months, ferritin injection. × 24,000.

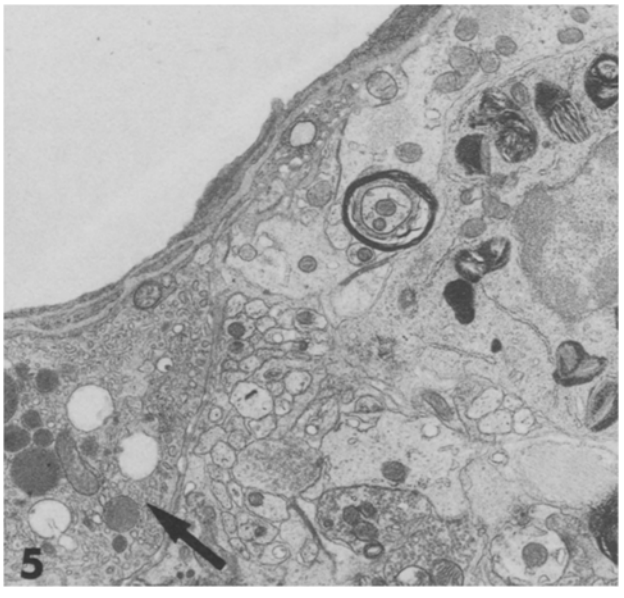


Figure 5. A general view of a region in the vicinity of, but not very close to a cerebral hemorrhage. At the left hand, the FGP with a normal appearance is seen (arrow). A migrating macrophage containing a lot of cell debris appears at the right hand. Rat, 36 months, spontaneous hemorrhage. × 8300.

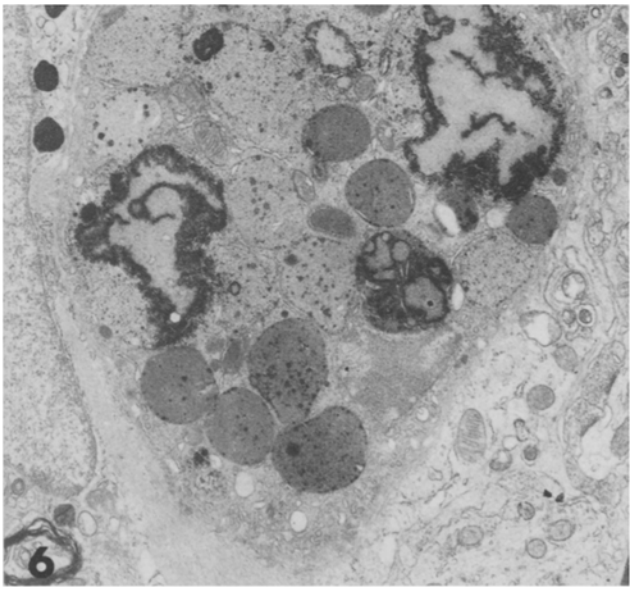
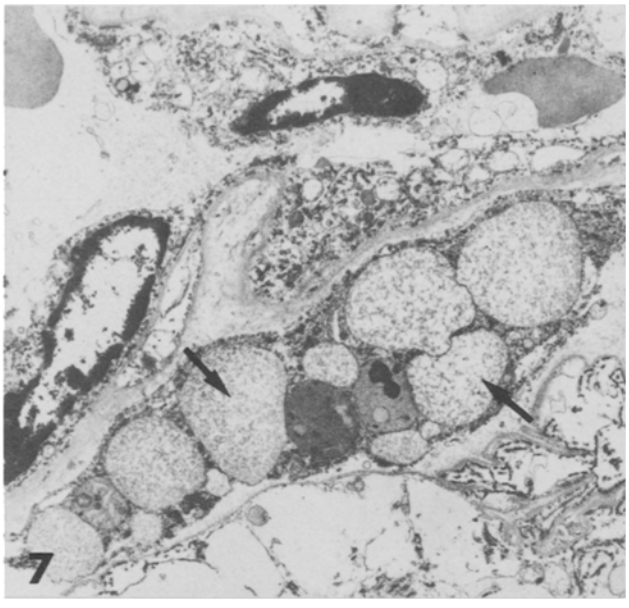
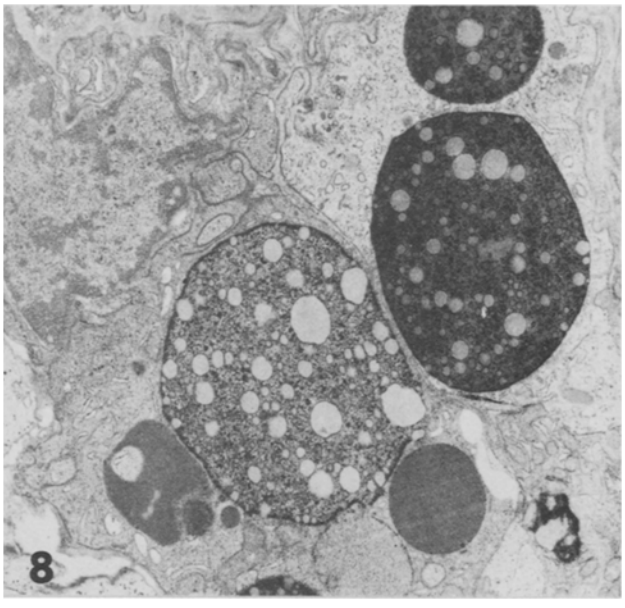


Figure 6. FGP closer to the bleeding region. Some small residual bodies and lipoidal substances with irregular shape are observed. Rat, 36 months, spontaneous hemorrhage. × 8300.





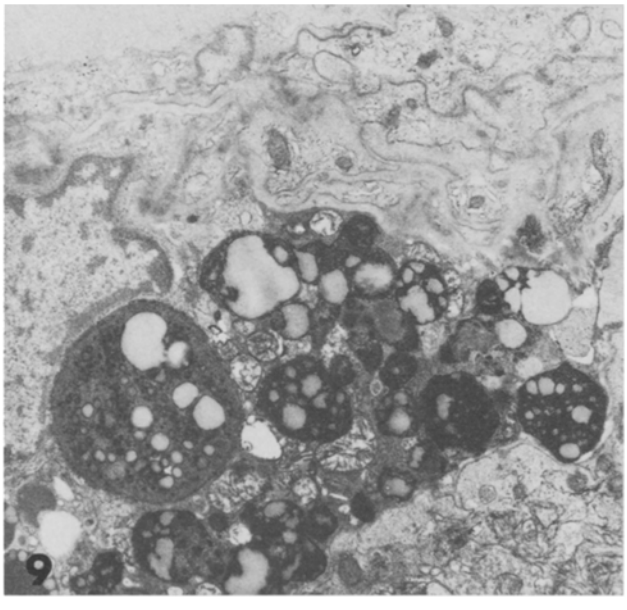


Figure 7. Pale and dotted inclusion bodies are seen in the cytoplasm of FGP (arrows). The cytoplasmic organelles are poorly developed. Human, 2 years old, lipidosis. × 6700.

Figure 8. Inclusion bodies with larger and smaller vacuoles are present. The electron opacity of inclusion bodies is heterogeneous. Human, 15 years old, glioma. × 7500.

Figure 9. The FGP is filled up with various kinds of inclusion bodies, some of which are fused together. Human, 54 years old, aneurysm. × 7500.

compared with those in figure 2, the bodies looked less dense and had an irregular shape. The occurrence of vacuolation and reduction in electron opacity of inclusion bodies seem to be caused by cerebral edema and the breakdown of neural tissue by the growing glioma.

In older individuals, the inclusion bodies became irregular in shape and the small ones coalesced with each other (fig. 9). Pale blebs increased in the inclusions and formed large spotted bodies. The findings may reflect a spontaneous regression of neurons and an increase of waste products in the central nervous system with aging.

Discussion. The FGP of young animals and humans were provided with homogeneous, dense inclusion bodies. They were often situated in the bifurcating regions of small cerebral vessels. The reason for their location at this site is not clear. The detailed observation on the branching regions suggested a morphological specificity of this area. Although nerve endings were not observed close to this region, the specific morphology of high endothelium and irregularly arranged smooth muscle cells seemed to contribute to the regulation of peripheral blood flow and to afford favorable conditions for the uptake of cerebral waste products by FGP. As previously reported[3,4], horseradish peroxidase (M.W. 40,000) and lipoidal substance were easily incorporated into FGP. However, it was not yet clear whether the proteinous substances with high molecular weight, solid particles and cell debris could be taken up by FGP or not.

In order to clarify this point, ferritin (M.W. 900,000) and India ink were injected into rats with or without cerebral hemorrhages. As shown above, the injected substances were ingested into the inclusion bodies of FGP. Observations on brain hemorrhage showed that the FGP serve as scavenger cells only for substances which can be transported through the cellular interstices of cerebral tissue. A particular type of enlarged and pale inclusion body occurred in the FGP of the patient with GM2 lipidosis.

In 1965, Uchimua et al.[7] reported that pericytes and adventitial cells in patients with gargoylism contained a lot of vacuoles. Presumably, their pericytes and adventitial cells might be identical to our FGP. The morphological changes in FGP of aged

human specimens were similar to those found in rats. However, the honeycomb-like inclusion bodies often present in old rats' FGP were scarcely seen in old human specimens, although pale lipoidal blebs increased in the inclusion bodies. It is possible that the honeycomb-like structures in rat FGP do not always imply degeneration of the FGP as suggested by Sturrock[8]. According to our unpublished data, these honeycomb structures were rich in lipase and lacking in acid phosphatase, whereas typical dense inclusion bodies in FGP were rich in acid phosphatase, but lacking in lipase.

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Transplantation of human cortex with Alzheimer's disease into rat occipital cortex; a model for the study of Alzheimer disease[1]

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Summary. Senile dementia of the Alzheimer type (SDAT) is a major problem in the human senescent population. As this pathology cannot be reproduced in animals, research into its development is greatly impeded. The technique of implantation of the nervous tissue has been utilized in order to establish an animal model and to test the possible existence of a transmissible agent. When human temporal cortex with Alzheimer's disease is implanted in the occipital cortex of 7-week-old rats, human cerebral tissue containing abundant tangles induces in the receiver cortex a reactive fibrous gliosis. In the processes of the astrocytes, twisted filaments are evident among bundles of normal filaments. These alterations could be induced by the metabolising of abnormal filament subunits or by some infectious agent introduced by the implant.

Senile dementia of the Alzheimer type (SDAT) is a major health problem in the human senescent population. Autopsy screening confirms that of all the pathologies seen in old age, SDAT is certainly the most common and it may account for as much as 80% of all dementias in the elderly[2].

The brains of patients with Alzheimer disease reveal severe abnormalities; neurofibrillary tangles, neuritic or senile plaques, granulo-vacuolar degeneration, Hirano bodies and nerve cell loss. All these alterations are observed during normal brain aging but they are more abundant in SDAT, and may invade all the regions of the brain. However they occur predominantly in the frontal and temporal cortex and in the hippocampus[3].

The most striking morphological impairment in SDAT is the neurofibrillary tangles. These thickened fibers can be visualized by silver impregnation or by polarized light after Congo red staining. At the ultrastructural level they are characterized by paired helical filaments (PHF) crossing one another approximatively every 80 nm. The nature of the material composing the PHF is still uncertain.

The causes of SDAT are still enigmatic; genetic predisposition[4], exogenous toxin[5] and a slow latent virus[6] are suspected. As cholinergic neurons appear to be particularly altered[7], patients have been treated by choline precursors but these trials have been unsuccessful[8].

A major problem in planning research into the process of SDAT is the absence of a fully satisfactory animal model. This pathology cannot be reproduced in animals, and occurs as a natural disease in man only. Several attempts have been performed to simulate Alzheimer disease experimentally in animals. Injections of aluminium salts in rabbits induce neurofibrillary tangles[9], as does administration of alcohol in spinal neurons[10]. The scrapie agent induces in the mouse cortex senile plaques similar to the human ones[11].

However, the results obtained in the experimental models, produced by means of unconventional agents, are much more diverse than expected, and difficult to interpret.

Some previous work performed with the spinal ganglion neurons has shown that the cytoskeleton was dramatically impaired in the senescent rat[12]. In some cases, one of the alterations observed is that the pericaryon can contain local accumulations of filamentous structure similar to the human PHF. The occurrence of these impairments has been also mentioned in one individual of another strain of rats, the normotensive Kyoto[13]. On the basis of these results, our strain of rats has

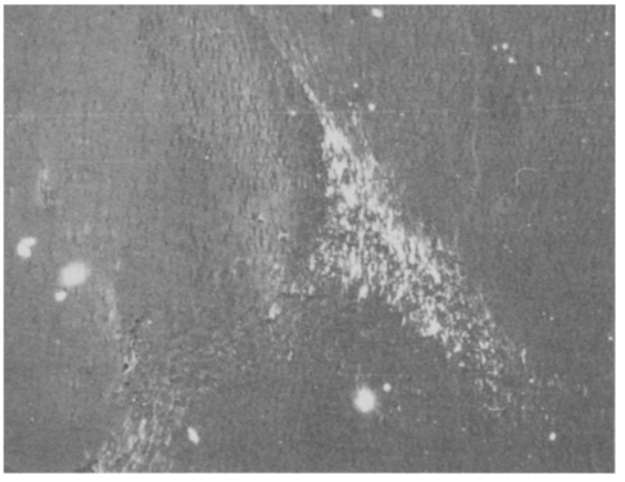

Figure 1. Implant of human cortex with Alzheimer's disease in rat cortex; tangles are visualized by polarized light after Congo red staining.